UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/673,786	09/30/2003	Valery Zavenovich Akhverdian	US-115	7880

38108 7590 07/15/2008

CERMAK & KENEALY LLP
ACS LLC
515 EAST BRADDOCK ROAD
SUITE B
ALEXANDRIA, VA 22314

EXAMINER	
RAMIREZ, DELIA M	
ART UNIT	PAPER NUMBER
1652	
MAIL DATE	DELIVERY MODE
07/15/2008	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte VALERY Z. AKHVERDIAN, EKATERINA A. SAVRASOSA, ALLA M. KAPLAN, ANDREY O. LOBANOV, and YURI I. KOZLOV

Appeal 2008-3648
Application 10/673,786
Technology Center 1600

Decided: July 15, 2008

Before RICHARD E. SCHAFER, SALLY GARDNER LANE, and MICHAEL P. TIERNEY *Administrative Patent Judges*.

LANE, *Administrative Patent Judge*.

DECISION ON APPEAL

I. STATEMENT OF THE CASE

The appeal is from a Final Rejection of claims 12, 15-16, 19, and 21-24. 35 U.S.C. § 134(a). We have jurisdiction under 35 U.S.C. § 6(b). We AFFIRM.

The application was filed September 30, 2003. It was published on July 8, 2004. The real party in interest is said to be Ajinomoto Co., Inc. (App. Br. 3).

The Examiner relied on the following references:

Name	Number	Date
Debabov	5,175,107	Dec. 29, 1992
Kishino	6,319,696	Nov. 20, 2001
Katsumata	EP 0219027	Apr. 22, 1987
Edwards	WO 87/00202	Jan. 15, 1987

Appellants did not argue against the prior art status of any of these references.

Appellants appealed the rejection of claims 12, 15-16, 19, and 23, all the pending claims, under 35 U.S.C. § 103(a) over the combination of the teachings of Katsumata, Debabov, Edwards, and Kishino. Appellants did not argue separately for the patentability of any of the rejected claims. We review claim 12 as a representative claim. *See* Bd. R. 41.37(c)(1)(vii).

II. FINDINGS OF FACT

The record supports the following findings of fact as well as any other findings of fact set forth in this opinion, by at least a preponderance of the evidence.

1. Claim 12 recites:

A method for producing L- threonine comprising:
A) cultivating in a culture medium an L-threonine-producing *Escherichia coli* bacterium, wherein the bacterium has been modified to increase the expression of:
i) the aspartate aminotransferase gene encoding the protein comprising the amino acid sequence of SEQ ID NO. 2,
ii) the *Escherichia coli thrA* gene which codes for an aspartokinase homoserine dehydrogenase I which is resistant to feedback inhibition by threonine,
iii) the *Escherichia coli thrB* gene,
iv) the *Escherichia coli thrC* gene, and

v) the *Escherichia coli rhtA* gene,

wherein said expression of said genes is increased by a method selected from the group consisting of increasing the copy number of said gene and placing said gene under the control of a potent promoter, and

B) collecting the L-threonine from the culture medium.

(App. Br. 12, Claims Appendix)

2. Katsumata relates to "a process for producing L-lysine, L-threonine, or L-isoleucine . . . ." (Katsumata 1, ll. 28-29).

3. Katsumata teaches that the enzyme aspartate aminotransferase, referred to by Katsumata as EC 2.6.1.1 and "AAT," is "important in the production of amino acids such as lysine, threonine and isoleucine whose precursor is aspartic acid." (Katsumata 1, ll. 21-27).

4. Katsumata teaches producing L-lysine, L-threonine or L-isoluecine by "introducing a recombinant DNA comprising a gene coding for ASD or AAT and a vector DNA into a coryneform bacterium such as a microorganism of the genus Corynebacterium or Brevibacterium and culturing the bacterium in a medium." (Katsumata 1, ll. 27-33).

5. Example 2, part (1), of Katsumata describes the "[c]loning of a gene coding for AAT of Corynebacterium glutamicum." (Katsumata 17, l. 26, through 19, l. 19).

6. Example 2, part (4) of Katsumata describes "[p]roduction of threonine by Corynebacterium glutamicum ATCC 21660 containing pAT1," (Katsumata 20, l. 25, through 21, l. 13), wherein "pAT1" contains the cloned AAT gene (*see* Katsumata 19, ll. 17-19).

7. Katsumata states that the results of the studies on the production of threonine, provided in Table 5, "confirm that threonine-

producing ability is increased by the recombinant plasmid pAT1 containing the gene coding for AAT." (Katsumata 21, ll. 10-13).

8. Debabov addresses the problem of "low productivity and inadequate stability of the plasmid [pYN7] . . . ," which was previously used in *E. coli* to produce L-threonine. (Debabov col. 1, ll. 42-45).

9. Debabov teaches "[a] bacterial strain of *Escherichia coli* BKIIM B-3996, a producere [sic] of L-threonine, containing a recombinant plasmid pVIC40 . . . ." (Debabov abstract; *see also* Debabov claim 1).

10. Appellants' specification discloses that the "strain B-3996 is deficient in the *thrC* gene . . . . has a mutation in *rhtA* gene . . ." and "harbors the plasmid pVIC40 which had been obtained by inserting *thrA*BC* operon including mutant *thrA* gene encoding aspartokinase homoserine deyhdrogenase 1 which is substantially desensitized feedback inhibition by threonine into RSF1010-derived vector." (Spec. 11-12, ¶ [0050]).

11. Edwards relates to "[c]omposite plasmids containing multiple genes in transcriptional units. These composite plasmids are useful for the production of amino acids, particularly aromatic amino acids." (Edwards abstract).

12. Threonine is not an aromatic amino acid.

13. Edwards teaches incorporating the gene for aspartate amino transferase, abbreviated as "*aspC*," into an *E. coli* host ("HW-77"), with a resulting increase in aspartate aminotransferase activity. (Edwards 36-37; Table 6).

14. The sequence of the *aspC* gene disclosed by Edwards is the same as Appellants' SEQ ID NO: 1 and encodes the polypeptide of

Appellants' SEQ ID NO: 2. (*See* Edwards 21, chart 6; *see also* comparison at Ans. 5-9).

15. Kishino relates to "[a] microorganism, which has an ability to produce L-amino acid, especially . . . L-threonine, . . . in which a phosphoenolpyruvate-producing ability is enhanced . . . ." (Kishino abstract).

16. Kishino teaches that the "means for increasing the amount of expression of the pps gene [which encodes phosphoenolpyruvate synthase] in the microbial cells include, for example, a method to increase a copy number of the pps gene in the microbial cells, as well as a method to enhance a transcriptive activity of a promoter for the pps gene." (Kishino col. 3, ll. 35-39).

17. Kishino teaches that the microorganisms "preferably used for L-threonine include, for example, *Escherichia coli* VKPM B-3996 . . . ." (Kishino col. 4, ll. 7-8).

18. Kishino teaches that "[i]n order to achieve efficient expression of PPS, it is also preferable to use a promoter which functions in the microorganism, such as lac, trp, or P_L." (Kishino col. 5, ll. 42-44).

19. Appellants' specification identifies the *lac*, *trp*, and P_L promoters, among others, as "potent promoters." (Spec. 10, ¶ [0043]).

III. ISSUES

The issue is whether the Appellants have shown that the Examiner erred in rejecting claims 12, 15-16, 19, and 23 under 35 U.S.C. § 103(a) over the combination of the teachings of Katsumata, Debabov, Edwards, and Kishino.

IV. LEGAL PRINCIPLES

To determine whether subject matter would have been obvious, "the scope and content of the prior art are to be determined; differences between the prior art and the claims at issue are to be ascertained; and the level of ordinary skill in the pertinent art resolved . . . . Such secondary considerations as commercial success, long felt but unsolved needs, failure of others, etc., might be utilized to give light to the circumstances surrounding the origin of the subject matter sought to be patented." *Graham v. John Deere Co. of Kansas City*, 383 U.S. 1, 17-18 (1966).

The Supreme Court has noted that

> [w]hen a work is available in one field of endeavor, design incentive and other market forces can prompt variations of it, either in the same filed or a different one. If a person of ordinary skill can implement a predictable variation, § 103 likely bars its patentability. For the same reason, if a technique has been used to improve one device, and a person of skill in the art would recognize that it would improve similar devices in the same way, using the technique is obvious unless its actual application is beyond his or her skill.

KSR Int'l Co. v. Teleflex Inc., 127 S.Ct. 1727, 1740 (2007). Furthermore, "when a patent claims a structure already known in the prior art that is altered by the mere substitution of one element for another known in the field, the combination must do more than yield a predictable result." *Id.*

V. ANALYSIS

Claim 12 recites:

> A method for producing L- threonine comprising:

A) cultivating in a culture medium an L-threonine-producing *Escherichia coli* bacterium, wherein the bacterium has been modified to increase the expression of:

i) the aspartate aminotransferase gene encoding the protein comprising the amino acid sequence of SEQ ID NO. 2,

ii) the *Escherichia coli thrA* gene which codes for an aspartokinase homoserine dehydrogenase I which is resistant to feedback inhibition by threonine,

iii) the *Escherichia coli thrB* gene,

iv) the *Escherichia coli thrC* gene, and

v) the *Escherichia coli rhtA* gene,

wherein said expression of said genes is increased by a method selected from the group consisting of increasing the copy number of said gene and placing said gene under the control of a potent promoter, and

B) collecting the L-threonine from the culture medium.

(FF[1] 1). Katsumata teaches a process for producing L-threonine (FFs 2-3), but employs a different bacteria than the one claimed. Specifically, Katsumata teaches introducing a gene for aspartate aminotransferase cloned from *Corynebacterium glutamicum* (FFs 4-5) into this same species (FF 6) to achieve increased threonine-producing ability (FF 7). While Katsumata differs from the claimed method by using a different bacterium, it taught those in the art to introduce a gene encoding an aspartate aminotransferase into bacteria to increase L-threonine production.

The bacteria strain recited in the claimed method was known in the art. Debabov addresses the problem of "low productivity and inadequate stability of the plasmid [pYN7] . . . ," which was previously used in *E. coli* to produce L-threonine (FF 8), by teaching "[a] bacterial strain of *Escherichia coli* BKIIM B-3996, a producere [sic] of L-threonine, containing a recombinant plasmid pVIC40 . . . ." (FF 9). As indicated in

[1] Finding of Fact.

Appellants' specification, this strain, B-3996, has a deficient thrC gene, a mutation in the rhtA gene and "harbors the plasmid pVIC40 which had been obtained by inserting *thrA*BC* operon including mutant *thrA* gene encoding aspartokinase homoserine deyhdrogenase 1 which is substantially desensitized feedback inhibition by threonine into RSF1010-derived vector." (FF 10). Thus, Debabov teaches L-threonine production using a bacterial strain that meets the elements of having been modified to increase expression of the *Escherichia coli thrA* gene that is resistant to feedback inhibition by threonine, the *Escherichia coli thrB* gene, the *Escherichia coli thrC* gene, and the *Escherichia coli rhtA* gene, as recited in Appellants' claim.

Kishino relates to microorganisms used to produce L-amino acids, including L-threonine, by enhancing the phosphoenolpyruvate-producing ability of the microorganism (FF 15). Although Kishino employs a gene for a different enzyme than that claimed, it teaches increasing the copy number of the gene in the microbial cells (FF 16) and using promoters such as lac, trp, or P_L (FF 18), which are "potent promoters" (FF 19). Furthermore, Kishino teaches using these techniques to produce L-threonine in *Escherichia* coli VKPM B-3996 (FF 17), which exhibits the claimed elements (FF 10).

From the combination of Katsumata, Debabov, and Kishino, which all relate to increasing L-threonine production, those in the art would have understood that genes can be introduced into the *E. coli* B-3996 strain, with increased copy number or under the control of potent promoters, to produce L-threonine.

Edwards relates to plasmids that "are useful for the production of amino acids, particularly aromatic amino acids." (FF 11). Edwards does not discuss threonine production and threonine is not an aromatic amino acid. (FF 12). Edwards does teach introduction of the aspartate aminotransferase gene into *E. coli*, with increased aspartate aminotransferase activity. (FF 13). The *aspC* gene taught by Edwards is the same as Appellants' SEQ ID NO: 1 and encodes the same amino acids as Appellants' SEQ ID NO: 2. (FF 14). Thus, using the *E. coli* aspartate aminotransferase gene taught in Edwards instead of the *Corynebacterium glutamicum* aspartate aminotransferase gene taught in Katsumata, with the specific *E. coli* strain taught in Debabov and the additional elements taught in Nishino, provides the elements of the claimed method.

The Examiner asserted that those in the art would have combined the cited references because

> (1) Katsumata et al. teach the production of L-threonine by culturing a microorganism transformed with a vector comprising the endogenous gene encoding aspartate aminotransferase so that the level of aspartate aminotransferase is increased in that microorganism, (2) Kishino et al. teach that high threonine producers, such as the strain of Debabov et al., are preferred strains for L-threonine production, (3) the gene taught by Edwards et al. is the *E. coli* aspartate aminotransferase gene and is the preferred aspartate aminotransferase gene to express by the E. *coli* strain of Debabov et al. because it would not be recognized by the cell as foreign, (4) a low copy number vector or an inducible promoter such as lac, trp, or P_L [as taught in Nishino] would allow for better control of how much of the aspartate aminotransferase is produced and avoid intracellular instability, and (5) aspartate aminotransferase catalyzes the conversion of oxaloacetate to aspartate, which is a precursor of L-threonine.

(Ans. 10). Furthermore, the Examiner asserted that skilled artisans would have had a reasonable expectation of success because

> (1) cloning genes in low copy number vectors and placing genes under the control of inducible promoters such as lac, trp or P_L is well known and widely practiced in the art, (2) transformation of *E. coli* cells is well known and widely used in the art, (3) increasing the levels of aspartate aminotransferase should result in an increase in aspartate, which in turn should result in increasing levels of L-threonine, and (4) Katsumata et al. teach L-threonine production by culturing a microorganism wherein the endogenous aspartate aminotransferase levels are increased.

(Ans. 10-11). "When a patent claims a structure already known in the prior art that is altered by the mere substitution of one element for another known in the filed, the combination must do more than yield a predictable results." *KSR*, 127 S.Ct. at 1740. We agree that the combination of Katsumata, Debabov, Nishino, and Edwards presents a prima facie case for obviousness.

Appellants argued that "[i]t is not clear from the prior art . . . which type of aminotransferase is effective for increasing a production of L-threonine." (App. Br. 7). As evidence, Appellants provided Exhibit A, Marienhagen et al., "Functional Analysis of All Aminotransferase Proteins Inferred from the Genome Sequence of *Corynebacterium glutamicum*," *J. Bacteriol.*, vol. 187, pp. 7639-46 (2005) ("Exhibit A"), which they asserted demonstrates that "[m]any types of aminotransferase enzymes are known, and their substrate specificities are each different." (App. Br. 7).

Exhibit A, which was published in 2005, after Appellants' filing date in 2003, identifies only one *aspartate* aminotransferase. Thus, even though it may list many aminotransferases, we are not convinced that those in the

art would not understand from Katsumata that aspartate aminotransferase increases L-threonine production. (*See* FFs 2-7).

Appellants argued that neither Katsumata, Debabov, nor Kishino teach SEQ ID NO: 2. (App. Br. 7 and 8). But, Edwards does teach SEQ ID NO: 2. "Non-obviousness cannot be established by attacking references individually where the rejection is based upon the teachings of a combination of references," *In re Merck & Co., Inc.*, 800 F.2d 1091, 1097 (Fed. Cir. 1986), thus, we do not find Appellants' arguments persuasive.

Appellants argued that Edwards does not cure the "deficiency" of Katsumata because "one of ordinary skill in the art would not have been able to determine or deduce that increasing the expression of the aspartate amino transferase gene of *E. coli* would be effective for producing L-threonine, since the production pathway of L-phenylalanine is completely different from that of L-threonine." (App. Br. 7). We are not convinced by this argument because Katsumata taught those of skill in the art to use aspartate aminotransferase for increased L-threonine production, while Edwards provides the specific sequence recited in the claim. It is the combination of these references that renders the claimed method obvious. *See Merck, supra.*

Appellants argued that "[i]t would not have been expected by the ordinarily skilled art worker that an additional increase in threonine production would result by increasing the expression of the gene depicted in SEQ ID NO: 2, because threonine production is *already* optimized at a high level in a bacterium with increased expression of the *thrA*, *thrB*, *thrC*, and *rhtA* genes." (App. Br. 8). We disagree. Appellant has not directed us to any specific teaching in Debabov, or any other prior art, that production of

L-threonine in the recited *E. coli* strain could not be increased further. Nor has the Appellants directed us to other evidence in support of its position regarding what one skilled in the art would have expected at the time of the invention. Attorney argument is not evidence. "Argument of counsel cannot take the place of evidence lacking in the record." *Meitzner v. Mindick*, 549 F.2d 775, 782 (CCPA 1977). Instead, we agree that those of skill in the art would learn from Katsumata and Debabov that there are different ways to increase L-threonine production and that these different techniques could be combined to further increased L-threonine production.

Appellants also argued that

> the *aspC* gene (SEQ ID NO: 2) encodes an enzyme which catalyzes the synthesis of aspartic acid from oxaloacetic acid. However, one of ordinary skill in the art would know that even if expression of the *aspC* gene is increased, the production of threonine would not also increase unless the supply of aspartic acid runs short in the whole pathway of threonine synthesis. The shortage of aspartic acid will occur if the synthesis reaction of aspartic acid from oxaloacetic acid is the rate-limiting step. However, one of ordinary skill in the art would not have known which reaction is the rate-limiting step in threonine synthesis. Therefore, the additional increase in threonine as a result of the combination of increasing expression of the aspC gene with increasing expression of the thrA, thrB, thrC and rhtA genes is completely unexpected.

(App. Br. 8-9). We note at the outset that Appellant did not direct us to evidence to support these arguments, either in the prior art or by one who could testify about the knowledge of those in the art at the time of Appellants' filing. *See Meitzner, supra.*

As we understand it, Appellants argument is that expression of the *aspC* gene would not be expected to result in increased L-threonine

production unless aspartic acid is limiting, which occurs when the synthesis of aspartic acid by oxaloacetic acid is a rate-limiting step. But, Appellants have not demonstrated that this situation is different in the *C. glutamicum* used in Katsumata and the *E. coli* strain used in Debabov. Appellants have not provided sufficient support to convince us that those of skill in the art would have expected the phenotype of the *E. coli* strain taught in Debabov to cause any difference in the results reported in Katsumata. Thus, because increased expression of aspartate aminotransferase increased threonine production in *C. glutamicum*, as reported in Katsumata, Appellants have not convinced us that those in the art would not expect it to also increase production of L-threonine in the *E. coli* strain taught in Debabov.

Appellants further argued, in the Reply Brief, that "the Examiner's assertion that the threonine synthetic pathways of *C. glutamicum* described in the prior art would be predicative [sic] of the same pathways in *E. coli* is incorrect." (Reply Br. 4). Specifically, Appellants asserted the following differences between the pathways in each:

> For example, in the regulatory pathways and biosynthesis of lysine and threonine, whereas only one type of aspartokinase is required in *C. glutamicum,* three different isozymes of aspartokinase are required for the same function in *E. coli*. As a result, the system of biosynthesis and regulation in *E. coli* is far more complex, as further demonstrated by the fact that the one aspartokinase of *C. glutamicum* is inhibited by the concerted feedback of both lysine and threonine, whereas the three types of isozymes present in this same pathway in *E. coli* are each inhibited separately by lysine, threonine, and methionine, respectively.
>
> To further show the differences between these pathways in these two microorganisms, expression of the aspartokinase gene of *C. glutamicum* is *not* repressed by lysine, threonine, or

> methionine, whereas expression of each isozyme gene in *E. coli* is repressed by all of these amino acids. Also, *C. glutamicum* has only one isozyme of homoserine dehydrogenase, whereas *E. coli* has two isozymes. which work as bifunctional enzymes, homoserine dehydrogenase/aspartokinase, further adding to the complexity of the pathway.

(Reply. Br. 4-5). Again, Appellants did not direct us to evidence supporting these assertions. *See Meitzner, supra*. Furthermore, Appellants did not provide any explanation of how any of these observations impact the teachings of Katsumata, Debabov, Kishino, or Edwards. For example, Appellants did not explain if these observations relate to any difference in the activity of aspartate aminotransferase in *C. glutamicum* versus *E coli*. Thus, even if these observations were supported by evidence, we do not find them persuasive that those of skill in the art would not have combined the teachings of Katsumata, Debabov, Kishino, and Edwards to perform the claimed method.

Accordingly, Appellants have not shown that the Examiner erred in rejecting claim 12, 15-16, 19, and 23 under 35 U.S.C. § 103(a) over Katsumata, Debabov, Kishino, and Edwards.

VI. ORDER

Upon consideration of the record and for the reasons given, the Examiner's rejection of claims 12, 15-16, 19, and 23 under 35 U.S.C. § 103(a) over the combination of the teachings of Katsumata, Debabov, Edwards, and Kishino is AFFIRMED.

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a).

AFFIRMED

rvb

CERMAK & KENEALY LLP
ACS LLC
515 East Braddock Road
Suite B
Alexandria, VA 22314